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ANTIBACTERIAL ACTIVITY OF THE PENICILLINS

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Penicillin, the first antibiotic of clinical importance to be discovered, is in many ways the best. Against most pathogenic species of Gram-positive bacteria and Gram-negative cocci a concentration of 0.02 μg./ml.† or less inhibits growth and a concentration not much higher is actively bactericidal. Moreover, apart from hypersensitivity reactions, penicillin is probably the least toxic of the antibiotics in clinical use.

Disadvantages of Penicillin

From the onset of its introduction into clinical medicine, however, penicillin presented two obvious disadvantages as a therapeutic agent—namely, instability and extremely rapid excretion via the kidney tubules. With regard to the first, penicillin in aqueous solution is rapidly inactivated by many agents, including acids and alkalis, oxidizing agents, heavy metals, alcohols, some forms of synthetic rubber, and the enzymes of certain bacteria. The importance of acid instability was appreciated at once, since it precluded oral administration, except in the newborn, and this fact, together with the rapid excretion, made it necessary to administer penicillin by frequent (four-hourly) intramuscular injection or continuous intramuscular or intravenous transfusion.

A bacterial enzyme inactivating penicillin and named penicillinase was isolated in 1940 by Abraham and Chain from a strain of *Escherichia coli*. At first this was simply regarded as an interesting mechanism to which some species of bacteria, such as coliform bacilli, regarded from the first as penicillin-resistant, owed their resistance. But the full clinical importance of the enzyme was appreciated only when, following the widespread clinical use of penicillin, strains of *Staphylococcus aureus* which produced penicillinase triumphantly supplanted less adaptable strains in hospital communities all over the world.

In the 20 years since the introduction of penicillin much has been done to mitigate the disadvantages. First of all, excretion can be delayed by the use of insoluble, and therefore slowly absorbed, salts of benzylpenicillin—for example, procaine (Sullivan *et al.*, 1948; Young *et al.*, 1949; Wayne *et al.*, 1949) and benzathine (Fletcher and Knappett, 1953)—or by the simultaneous administration of a drug, such as probenecid ("benemid"), which blocks excretion by the kidney tubules (Boger *et al.*, 1950). Recently immense strides have been made by the discovery that penicillins with different side-chains have different properties, some of which may improve their therapeutic efficiency at least for certain purposes. A large number of these are now available for clinical use, and since their antibacterial activity is by no means identical the present comparative study was undertaken.

New Penicillins Available

Production.—Several hundred different penicillins have now been isolated. In early studies they were obtained by adding different side-chain precursors to fermentations of the mould *Penicillium chrysogenum*, and in this way phenoxymethylpenicillin (penicillin V) was obtained; but recent discoveries on the penicillin nucleus have made it possible to produce new penicillins with relative ease. In 1958 Sheehan synthesized the nucleus of penicillin, 6-aminopenicillanic acid (see Fig. 1), but the process was complicated and the final yields were small. However, two discoveries at the Beecham Research Laboratories have made it possible, through the intervention of microbes, to produce 6-aminopenicillanic acid on a large scale. The first was

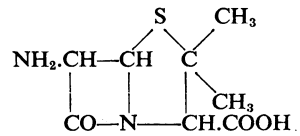


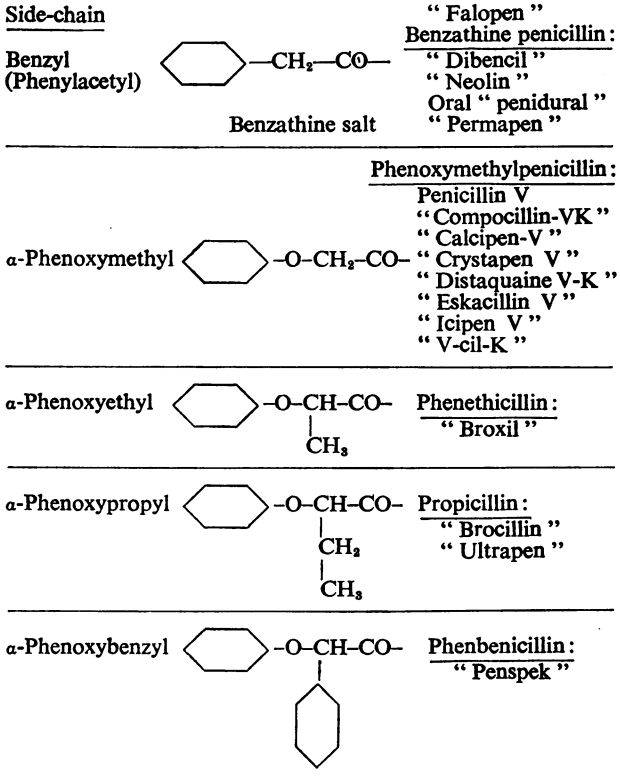
FIG. 1.—6-aminopenicillanic acid.

the discovery that it could be isolated from fermentations of *P. chrysogenum* to which no side-chain precursor had been added (Batchelor et al., 1961), and the second that certain species of bacteria produce enzymes referred to as amidases, which catalyse the removal of the side-chain from various penicillins, thus leaving the free nucleus (Rolinson *et al.*, 1960).

Acid-resistant Penicillins.—The acid-resistant penicillins available for oral administration for treatment of the usual penicillin-sensitive infections are shown in Fig. 2. Penicillins with a side-chain consisting of α-phenoxymethyl, α-phenoxyethyl, α-phenoxypropyl, or α-phenoxybenzyl are all relatively resistant to acid as compared with benzylpenicillin. Two preparations of

*Working with a full-time grant from the Medical Research Council.

†1 unit=0.6 μg., or 1 μg.=1.67 units.

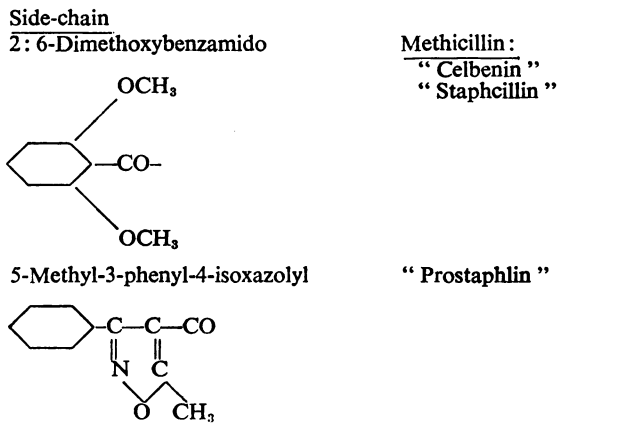


(Ampicillin and prostaphlin)

FIG. 2.—Acid-resistant penicillins.

the latter have, however, been recommended for oral use—namely, tablets with a protective coating insoluble at a *p*H below 6.5 (" falopen ") and the benzathine salt (oral benzathine penicillin). " Prostaphlin " (see Fig. 3) and ampicillin (see Fig. 4) are also acid-resistant.

Penicillinase-resistant Penicillins.—Two penicillins which are highly resistant to staphylococcal penicillinase have now been reported and are shown in Fig. 3. The first of these, 2:6-dimethoxybenzamido penicillin, or methicillin, is freely available and has proved its value



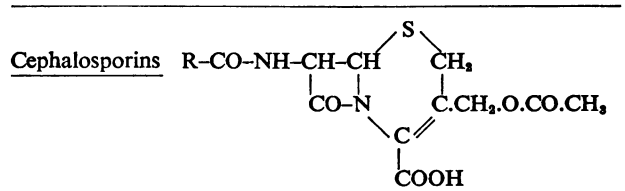


FIG. 3.—Penicillinase-resistant penicillins.

clinically. It is not resistant to acid and has therefore to be administered by injection. The second, 5-methyl-3-phenyl-4-isoxazolyl penicillin, has been recently described (Bunn and Amberg, 1961) and given the trade name prostaphlin. A series of isoxazolyl penicillins is also being studied at the Beecham Laboratories (Doyle *et al.*, 1961). The isoxazolyl penicillins are resistant to acid as well as to staphylococcal penicillinase, so they can be administered orally.

Another series of compounds resistant to staphylococcal penicillinase are the cephalosporins, which, as will be seen from Fig. 3, differ from the penicillins in that the nucleus consists of a fused β-lactam-dihydrothiazine ring instead of the β-lactam-thiazolidine ring system. The nomenclature here is somewhat confusing, since a *Cephalosporium* mould isolated from the sea near a sewage outfall of Sardinia gives rise to several antibiotics, two of which have been named cephalosporin N and C. In fact, the N compound is a true penicillin identical with the previously described penicillin, synnematin B, which has activity against Gram-negative bacilli (see Fig. 4). Cephalosporin C has the nucleus given in Fig. 3 and a side-chain similar to cephalosporin N. It is resistant to staphylococcal penicillinase, but has a low order of antibiotic activity. The nucleus of this antibiotic, which has been named

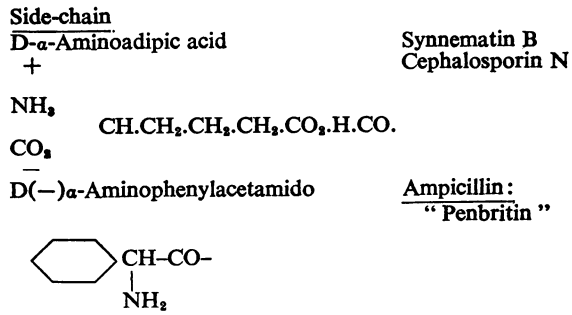


FIG. 4.—Penicillins active against Gram-negative bacilli.

7-aminocephalosporanic acid, has now been isolated by mild acid hydrolysis and a series of compounds are being prepared, some of which have much greater activity than cephalosporin C while retaining their resistance to staphylococcal penicillinase (for a review of this subject the reader is referred to Abraham and Newton, 1961).

Penicillins with Activity against Gram-negative Bacilli.—The first penicillin shown to have an increased activity against Gram-negative bacilli was synnematin B or cephalosporin N, referred to above, but this compound has less than 1% of the activity of benzylpenicillin against the Oxford staphylococcus. Recently ampicillin (a new penicillin with a D(–)α-aminophenylacetamido side-chain) (Fig. 4) has been prepared at the Beecham Research Laboratories which, like synnematin B, has increased activity against Gram-negative bacilli, but has an activity against Gram-positive organisms closer to that of benzylpenicillin.

Investigation of Antibacterial Activity

Materials and Methods

Antibiotics.—All antibiotics used were in powdered form ; the potassium salt of phenoxymethylpenicillin was supplied by Eli Lilly ; phenoxybenzylpenicillin (phenbenicillin) by Distillers Company ; ampicillin, methicillin, and the sodium salt of the three isoxazolyl penicillins

by the Beecham Research Laboratories; and phenylacetyl-amino-cephalosporanic acid thiouronium (PAT) by Glaxo Laboratories.* All the compounds were dissolved in water for use, and solutions of the appropriate concentrations were readily obtained at room temperature, except with cephalosporin PAT. The latter could be dissolved only by heating to 56° C., and even then only in a concentration of 250 μg./ml. Moreover, solutions tended to precipitate when cool, so that the minimum inhibitory concentrations obtained with this compound are subject to error.

Bacteriostatic Tests.—These were carried out by preparing serial doubling dilutions of antibiotic in nutrient agar and inoculating with a standard 1-mm. loopful of an overnight broth culture, diluted and undiluted as stated in results. Laked or heated blood was added for those species requiring it.

Bactericidal Tests.—These were carried out by the technique described by Chabbert (1957) (see also Chabbert and Patte, 1960). The essentials are as follows: strips of blotting-paper soaked in a solution of the antibiotic to be tested are placed on the surface of nutrient-agar plates and left overnight at 37° C. to permit diffusion of antibiotic, after which they are removed. A tambour of "cellophane" is uniformly seeded by flooding with an overnight broth culture diluted about 1 in 10 and then laid on the surface of the agar. After drying, the dish is incubated at 37° C. for six hours, at which time the tambour is transferred to a fresh Petri dish containing nutrient agar without antibiotic. (For full details see Garrod and Waterworth, 1962.)

Bacteriostatic Activity

Highly Sensitive Species.—The minimum inhibitory concentration of the eight penicillins and cephalosporin PAT for various bacterial species are given in Table I. Against the four species of Gram-positive bacteria and *N. gonorrhoeae*, the minimum inhibitory concentration of benzylpenicillin ranged from 0.06 to 0.007 μg./ml.; the corresponding figures for phenoxymethylpenicillin were 0.03 to 0.007 μg./ml.; none of the other compounds was as effective. Against *N. meningitidis*, benzylpenicillin had a similarly high activity, but that of phenoxymethylpenicillin was only one-quarter that of benzylpenicillin, and similar to that of ampicillin. In tests with *N. catarrhalis* ampicillin was the most active compound and inhibited six of the eight strains tested in a concentration of 0.015 μg./ml. Benzylpenicillin inhibited all eight strains in a concentration of 0.03 μg./ml. All the other compounds were much less active.

Less Sensitive Species.—With the Gram-negative bacilli, ampicillin was in all cases the most active agent, with an activity four to eight times that of benzylpenicillin. All the other compounds were much less effective. The minimum inhibitory concentration of ampicillin (in μg./ml.) for various species was as follows: *H. influenzae* 0.125 to 0.5; *Salmonella* spp. 0.25 to 2; *Shigella* spp. 1 to 8; *E. coli* 2 to 8. Most strains of the *Proteus* group were highly resistant, but those strains of *Pr. mirabilis* which did not produce penicillinase were inhibited by from 2 to 8 μg. of ampicillin per ml.

Antistaphylococcal Activity of Penicillinase-resistant Penicillins

Bacteriostatic Test with a Small Inoculum.—Methicillin and two isoxazolyl penicillins were tested against 125 penicillinase-producing strains of *Staph. aureus*, the cephalosporin against 124 strains, and a third isoxazolyl penicillin against 23. The inoculum used was one standard 1-mm. loopful of a 1 in 100 dilution of an overnight broth culture. The results are shown in Table II, from which it will be seen that the growth of all 125 strains

*None of the isoxazolyl penicillins or the cephalosporin is yet generally available.

TABLE I.—*Minimum Inhibitory Concentration of Eight Penicillins and a Cephalosporin (Agar-plate dilution technique)*

	No. of Strains	Benzyl-penicillin	Phenoxy-methyl-penicillin	Phenbeni-cillin	Ampicillin	Methicillin	Isoxazolyl Penicillins			Cephalo-sporin PAT*
							Methylphenyl	1577	1621	
I. Highly Sensitive Species										
Staph. aureus (sens.) ..	12–36	−6 −5 −5	−6 −5 −5	−3 −3 −3	−5 −4 −3	−1 +1 +1	−3 −2 −1	−4 −3 −2	−4 −3 −2	−5 −3 −2
Str. pyogenes	10	−7 −6 −6	−7 −6 −6	−6 −5 −5	−6 −5 −5	−3 −3 −3	−5 −4 −4	−5 −5 −4	−4 −4 −4	−3 −2 −2
Dip. pneumoniae	16	−6 −6 −5	−6 −6 −5	−6 −6 −6	−5 −4 −4	−2 −2 −1	−4 −4 −3	−5 −4 −4	−3 −2 −2	−3 −3 −2
B. anthracis	13	−7 −7 −6	−7 −6 −6	−4 −4 −3	−5 −4 −4	−4 −3 −3	−2 −2 −2	−3 −2 −2	−1 −1 −1	+1 +2 +2
N. gonorrhoeae (sens.)	9	−6 −6 −5	−6 −5 −4	−3 −3 −2	−4 −3 −3	−5 −4 −0	−2 −1 −1	−2 −1 −1	−2 −1 −1	+4 +5 +5
N. gonorrhoeae (res.) ..	2	−3 and −1	−2 and +1	+1 and +2	−1 and 0	+1 and +2	>+2	>+2	>+2	>+5
N. meningitidis ..	7	−6 −5 −4	−4 −3 −3	−3 −3 −2	−4 −4 −2	−3 −2 1	−2 −1 −0	−3 −3 −2	−2 −1 0	+4 +5 +5
N. catarrhalis ..	8	−5 −5 −5	−4 −3 −3	−4 −3 −3	−6 −6 −4	−3 −2 −2	−2 −1 −1	−3 −3 −2	−2 0 +1	+2 +3 +4
II. Less Sensitive Species										
Str. faecalis	12	0 +1 +1	+1 +2 +2	+1 +2 +2	0 +1 +2	+4 +5 +5	+3 +4 +5	+3 +3 +4	+5 +5 +6	>+5
H. influenzae	15	−2 0 +2	+1 +2 +4	+1 +2 +3	−3 −2 −1	0 +1 +3	+3 +4 +5	+5 +6 +6	+3 +4 +5	+5 +5>+5
Salmonella spp. ..	7	+2 +3 +4	+6 +7 +8	+6 +7 +7	0 +1 +2	+8 +9 +9	+7 +7 +8	+9 +9 +9	+8 +8 +9	>+5
S. typhi ..	7	+1 +2 +2	+5 +6 +6	+6 +6 +6	−1 0 0	>+9	+6 +6 +6	+8 +8 +9	+8 +8 +9	>+5
Shigella spp.	9	+3 +4 +5	+6 +6 +8	+6 +6 +7	+1 +2 +3	+9 +9>+9	+6 +6 +9	+8 +8 +9	+6 +7 +9	>+5
Shigella shigae ..	1	+2	+5	+3	0	+7	+4	+5	+4	>+5
E. coli ..	11–15	+5 +6 +6	+6 +7 +7	+5 +6 +7	+1 +3 +3	+9 +9>+9	+7 +8 +8	+8 +8 +9	+7 +8 +9	>+5
Pr. mirabilis (a) ..	8	+3 +5 +5	+5 +7 +7	+7 +8 +8	+1 +2 +3	+7 +8 +9	+8 +8 +9	+9 +9>+9	+8 +9>+9	>+5
Pr. mirabilis (b) ..	6	>+9	>+9	>+9	>+8	+9 − >+9	>+9	+9 − >+9	+9 − >+9	>+5
Pr. vulgaris	7	+6 +9>+9	>+9	+7 +8 +9	+4 +6 +7	+8 +9>+9	+9>+9>+9	>+9	+9 +9>+9	>+5
,, *rettgeri*	6	>+9	>+9	+6 +6 +7	+5 +6 +9	+6 +8>+9	+6 +6 +8	+7 +7 +9	+6 +6 +8	>+5
,, *morgani*	6	>+9	>+9	+7 +7 +9	+5 +7>+9	+8 +9>+9	+8 +8 +9	+9 +9>+9	+9 +9>+9	>+5

0 denotes 1 μg./ml. Other figures are the $\log_2$ of the difference from this—i.e., +1=2 μg./ml., +2=4 μg./ml., +3=8 μg./ml., etc. −1=0·5 μg./ml. −2=0·25 μg./ml., −3=0·125 μg./ml., etc.
The first figure given is the *lowest*, the second (bold type) is the *usual*, and the third the *highest* minimum inhibitory concentration for the strain tested.
*+5 represents the highest concentration obtainable with cephalosporin PAT.
Proteus mirabilis (a) not penicillinase-producing. *Proteus mirabilis* (b) penicillinase-producing.

was inhibited by from 1 to 4 μg. of methicillin per ml. and 0.12 to 0.5 μg. of the other antibiotics tested per ml. When penicillin-sensitive strains are tested against these antibiotics the range of sensitivity is the same, although the average sensitivity is lower. Under the same conditions six naturally occurring methicillin-resistant strains were tested against the isoxazolyl compounds and the cephalosporin. These results are also shown in Table II, and it will be seen that the methicillin-resistant strains showed a slight degree of resistance to the other antibiotics.

Bactericidal Tests.—The bactericidal activity of methicillin, the isoxazolyl compounds, and cephalosporin PAT against a penicillinase-producing strain of *Staph. aureus* was compared with that of benzylpenicillin against a penicillin-sensitive strain by the cellophane transfer technique. The concentration of antibiotic solution used for the filter-paper strips was 50 μg./ml. for benzylpenicillin, 1,000 μg./ml. for methicillin, 600 μg./ml. for the isoxazolyl penicillins, and 250 μg./ml. for cephalosporin PAT. Typical results are shown in Figs. 5–8. All the antibiotics showed a zone of bactericidal activity, although this was smaller with cephalosporin PAT than with the penicillins. In all cases the zone was some distance from the site of maximum antibiotic concentration, thus indicating that there is an optimum bactericidal concentration and that an increase beyond this actually reduces the killing effect. This is a well-known phenomenon with benzylpenicillin, which is rapidly bactericidal for many strains of streptococci and staphylococci in a concentration of about 0.5 to 0.1 μg./ml. but kills much more slowly or not at all in concentrations of 1 μg./ml. or more (Garrod, 1945; Kirby, 1945; Eagle and Musselman, 1948; Eagle, 1951).

TABLE II.—*Minimum Inhibitory Concentrations of Penicillinase-resistant Penicillins for Penicillinase-producing Strains of Staph. aureus using a Small Inoculum*

Antibiotic	Total No. of Strains	No. of Strains with M.I.C. (μg./ml.)							M.I.C. of Six Naturally Occurring Methicillin-resistant Strains (μg./ml.)			
		0·06	0·12	0·25	0·5	1	2	4	0·5	1	2	4
Methicillin	125	0	0	0	1	23	89	12				
Isoxazolyl penicillins:												
Methylphenyl	125	0	8	78	39	0	0	0	0	5	1	0
1577	125	0	3	75	47	0	0	0	2	1	2	1
1621	23	7	4	9	3	0	0	0	2	4	0	0
Cephalosporin PAT	124	0	15	100	9	0	0	0	1	2	3	0

Effect of Size of Inoculum.—Six methicillin-sensitive penicillin-destroying strains were tested by a similar method for sensitivity to the same five antibiotics, but using both a small and a large inoculum—that is, one standard 1-mm. loopful of an overnight broth culture diluted 1 in 500 and undiluted. The average fold increase in resistance with the large inoculum is given in Table III. It will be seen that the difference is very slight with methicillin but higher with methylphenyl isoxazolyl penicillin and cephalosporin PAT. The two other isoxazolyl penicillins give intermediate results. These figures probably reflect the relative resistance of the compounds to penicillinase. (Under similar conditions the fold increase in resistance to benzylpenicillin would be from 10 to 100.)

TABLE III.—*Effect of Inoculum Size on Sensitivity of Penicillinase-producing Staph. aureus (Six Strains Tested). Tests Carried Out With Small and Large Inoculum*

Reaction to Methicillin	Average Fold Increase in M.I.C. with Large Inoculum				
	Methicillin	Isoxazolyl Penicillins			Cephalosporin PAT
		M.P.	1577	1621	
Sensitive	1·2	3·0	2·2	1·8	3·2
Resistant:					
Naturally occurring	12·0	12·7	10·7	9·0	14·7
Laboratory induced	1·5	7·3	7·3	3·0	7·3

Staphylococci showing Natural or Laboratory Induced Tolerance to Penicillinase-resistant Penicillins

A. Naturally Occurring Resistant Strains.

Incidence.—For the past 15 months all strains of *Staph. aureus* isolated in the routine laboratory of the hospital have been tested for sensitivity to methicillin by streaking on ditch-plates. During this time 4,017 strains have been tested; 1,078 of these came from infected patients, 334 of them being in surgical wards; the remaining strains came from nasal swabs and environmental studies. The incidence of methicillin-resistant strains in each group is given in Table IV. It will be seen that 2 to 3% of strains in each group were resistant to methicillin, but 83 of the 88 strains were

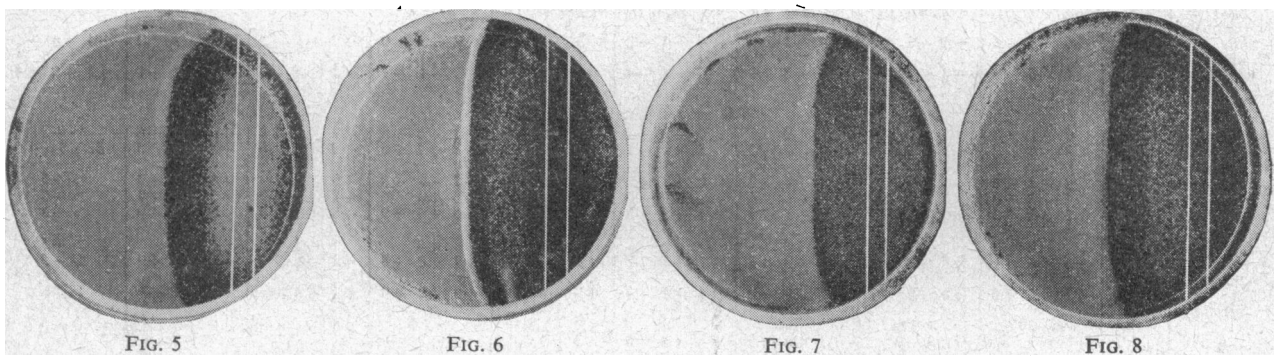


FIG. 5 FIG. 6 FIG. 7 FIG. 8

FIGS. 5–8.—Cellophane transfers seeded with *Staph aureus*, showing a zone of bactericidal activity (see text). White lines mark the original site of the blotting paper strip (that is, the site of maximum antibiotic concentration). Bacterial growth shows as light areas.

FIG. 5, Methicillin; FIG. 6, Isoxazolyl penicillin 1621; FIG. 7, Cephalosporin PAT. (These three seeded with penicillinase-producing strain). FIG. 8, Benzylpenicillin (seeded with penicillin-sensitive strain).

TABLE IV.—*Naturally Occurring Strains of Staph. aureus Showing Slight Resistance to Methicillin*

A. Incidence

	Total No.	Methicillin-resistant*	
		No.	% of Total
Strains from all sources	4,017	89	2·2
,, causing infections:			
All wards	1,078	20	2·0
Surgical ,, ...	334	10	3·0

B. Characteristics

Sensitivity to Other Antibiotics					Phage-type	No. of Strains
Penicillin	Streptomycin	Tetracycline	Chloramphenicol	Erythromycin		
R	R	R	R	S	53.75.77.	71
R	R	R	R	S	47.53.75.77.	3
R	R	R	S	S	53.54.75.77.	1
R	R	R	S	S	7.53.54.77.83.	1
R	R	R	S	R	83.	12

* The degree of resistance is dependent on the size of inoculum (see text), but is of a low order and would not necessarily prevent successful treatment with large doses of methicillin.

phage-type 53.75.77 or 83, both of which types were causing a number of infections in the surgical wards; 59 of the former type were isolated in May and June and 11 of the latter in October.

The *degree of resistance* of these strains to methicillin was in all cases of a low order, although they were all detected in routine laboratory tests in ditch-plates. A notable feature of the resistance was that it depended very much on the size of inoculum used (see Table III). This is not due to drug-destruction by a large inoculum, but results from the fact that these cultures consist of a mixed population of cells, and when plated on nutrient agar containing more than about 5 μg. of methicillin per ml. only a small proportion of cells grow, and these give rise to small atypical colonies. When a single colony is selected and retested once again only a proportion of cells grow in the presence of high concentrations of the antibiotic (see Knox and Smith, 1961; Barber, 1962).

All strains tested showed a corresponding increase in resistance to the isoxazolyl penicillins and to cephalosporin PAT, and with these compounds also the degree of resistance depended on the size of inoculum (see Table III).

B. Laboratory-induced Resistant Strains

As recorded elsewhere (Barber, 1961, 1962) strains of *Staph. aureus* can be rendered tolerant to methicillin, the isoxazolyl penicillins, and cephalosporin PAT by passage in their presence *in vitro*, although this does not happen readily. When strains are treated in this way the resulting drug-tolerant cultures usually resemble strains tolerant to benzylpenicillin in that they grow poorly in artificial medium and readily lose their resistance when passaged in the absence of the antibiotic. Penicillinase-producing strains, on the other hand, in some instances give rise to stable methicillin- or isoxazolyl-tolerant cultures, which in other respects, including mouse-virulence, resemble the parent strains.

With methicillin the degree of resistance of these strains was not greatly affected by the size of inoculum (see Table III) and with the penicillinase-producing cultures growth was equally good in the presence or absence of the antibiotic, within the limits of their resistance. With the other four antibiotics the size of inoculum had a considerable effect, and growth in the presence of the higher concentrations of the antibiotic tended to be rather less luxuriant than in its absence.

Cross-resistance with methicillin tends to be one way; that is to say, that passage in methicillin was usually followed by a similar increase in resistance to methicillin, the isoxazolyl penicillins, and cephalosporin PAT. On the other hand, strains passaged in the isoxazolyl penicillins or cephalosporin PAT showed only a slight increase in resistance to methicillin. Cross-resistance between the isoxazolyl penicillins and cephalosporin PAT occurred both ways.

Discussion and Conclusions

Choice of Oral Penicillin

The comparative antibacterial activity of benzylpenicillin and five acid-resistant penicillins is given in Table V. The figures are based on our findings together

TABLE V.—*Order of Antibacterial Activity of Benzylpenicillin and Five Acid-resistant Penicillins*

	Benzylpen.	Phenoxymethylpen	Phenethicillin (Phenoxyethylpen.)	Propicillin (Phenoxypropylpen.)	Phenbenicillin (Phenoxybenzylpen.)	Ampicillin
Staph. aureus (sens.) ..	1	1	3	4	6	5
Str. pyogenes	1	2	3	3	3	3
Dip. pneumoniae ..	1	2	5	4	2	6
B. anthracis	1	1	3	N.T.	5	3
N. gonorrhoeae ..	1	2	3	,,	5	3
N. meningitidis ..	1	2	4	4	4	2
N. catarrhalis	1	2	N.T.	N.T.	2	1
Str. faecalis	1	—	—	—	—	1
H. influenzae	2	—	—	—	—	1
Salmonellae	2	—	—	—	—	1
Shigellae ..	2	—	—	—	—	1
E. coli ..	2	—	—	—	—	1
Pr. mirabilis	2	—	—	—	—	1

N.T. = Not tested. — = Too inactive to be therapeutically useful.

with those of Garrod (1960). Against those species usually regarded as penicillin-sensitive, benzylpenicillin is unquestionably the most active, and it is second only to ampicillin against Gram-negative bacilli. Phenoxymethylpenicillin is a close second against the Gram-positive bacteria and *N. gonorrhoeae*, but ampicillin is about equally effective against *N. meningitidis* and more active against *N. catarrhalis*. Phenethicillin, propicillin, and phenbenicillin are appreciably less active. Against the Gram-negative bacilli ampicillin is about four times more active than benzylpenicillin and all three phenoxy compounds are much less active.

Against the inferior antibacterial activity of phenethicillin, propicillin, and phenbenicillin, it has been claimed that these are better absorbed from the alimentary tract and thus give higher blood levels than phenoxymethylpenicillin. There is some evidence to suggest that this is indeed the case, but as pointed out by the *British Medical Journal* (1962) proper comparative trials have not been undertaken. Moreover, chromatographic studies have shown that these penicillins are excreted in the urine in more than one active form. In view of the different antibacterial spectrum of different penicillins blood-level estimations which depend on a single organism may be fallacious. In any case, even if the reported blood levels for the phenoxy penicillins are taken at their face value, the higher levels of phenethicillin, propicillin, and phenbenicillin do no more than compensate for the greater activity of phenoxymethylpenicillin.

For infections with Gram-negative bacilli ampicillin is the penicillin of choice. As reported by Rolinson and Stevens (1961) it is highly active against *H. influenzae* and *Salmonella* species. It is moderately active against *Shigella* species and some strains of *E. coli* and *Pr. mirabilis*, but all these species produce penicillin-inactivating enzymes (Abraham and Chain, 1940; Bondi and Dietz, 1944; Housewright and Henry, 1947), and with the two latter there is considerable strain variation in sensitivity. Other species of *Proteus* and *Ps. pyocyanea* are highly resistant.

Penicillinase-resistant Penicillins

As recorded by Bunn and Amberg (1961) and Doyle *et al.* (1961) the isoxazolyl penicillins are resistant to staphylococcal penicillinase and to acid and have a higher antibacterial activity than methicillin against both penicillin-resistant staphylococci and penicillin-sensitive bacteria. We have confirmed the high antistaphylococcal activity in tests with 125 recently isolated strains of penicillinase-producing *Staph. aureus*. Unlike methicillin, however, the isoxazolyl penicillins were much less effective against a large than against a small inoculum, which reflects the fact that they are slightly less resistant than methicillin to staphylococcal penicillinase (Rolinson, personal communication).

Naturally occurring methicillin-resistant strains of *Staph. aureus* have been found in several laboratories (Jevons, 1961; Knox and Smith, 1961; Barber, 1961; Eriksen, 1961). These strains do not inactivate methicillin, and the degree of resistance is of a low order. They show a similar increase in resistance to the isoxazolyl penicillins. It is difficult to estimate their incidence since most strains belong to one or two closely related phage-types of group 3. It is of some interest that the first penicillin-resistant staphylococci recorded in hospitals in many parts of the world also belonged to one or two phage-types of this group (Barber and Whitehead, 1949; Rountree and Thomson, 1949; Fouace and Lutz, 1953; Wallmark, 1954; Fusillo *et al.*, 1954; Jackson *et al.*, 1954).

Drug-tolerant strains have also been isolated in the laboratory by passage of staphylococci in the presence of methicillin, the isoxazolyl penicillins, or cephalosporin PAT. Such strains are not readily obtained, but some pencillinase-producing strains of *Staph. aureus* develop a considerable degree of resistance without losing the cultural characteristics of the parent strain, and the resistance is stable. With the strains so far studied, however, the resistant variants grew poorly in the presence of high concentrations of the isoxazolyl penicillins.

It seems unlikely from these findings that drug-resistant strains will emerge during a single short course of treatment with these penicillins. On the other hand, if their use is widespread in wards where cross-infection is occurring drug-resistance might once more become a problem. For this reason, as well as the fact that these compounds are less active than other penicillins against most penicillin-sensitive bacteria, they should be reserved for the treatment of severe penicillin-resistant staphylococcal infection, and wherever possible such cases should be isolated.

Summary

A comparative study has been made of the bacteriostatic activity of eight penicillins and a cephalosporin against 154 strains of bacteria belonging to 19 different species.

Four of the penicillins and the cephalosporin are resistant to penicillinase. The bacteriostatic and bactericidal activity of these antibiotics were further studied in tests involving 137 penicillinase-producing strains of *Staph. aureus*, 12 of which showed varying degrees of resistance to methicillin.

On the basis of these findings, together with the comparative absorption of the compounds studied, the penicillin of choice for various types of infection is discussed.

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A new British Standard covering endotracheal tubes (B.S. 3487: 1962) has been published. The technical committee which drew up the standard included representatives of anaesthetists of Great Britain and Ireland, the Faculty of Anaesthetists of the Royal College of Surgeons, and the principal manufacturers of endotracheal tubes. The standard is split into three sections: general requirements for endotracheal tubes; oral (plain and cuffed) and nasal endotracheal tubes (Magill tubes) made of natural rubber; and reinforced endotracheal tubes with internally fitted exposed reinforcing helix. Copies of this standard may be obtained from the British Standards Institution, Sales Branch, 2 Park Street, London W.1. (Price 6s. each, postage extra to non-subscribers.)